REMARKS

Applicants have canceled claims 1-20 and added new claims 119-143. Support for the new claims may be found in originally filed claims 1-119 and in the specification, e.g., on page 12, lines 26-28, page 15, lines 21-23, page 15, line 27 to page 16, line 4, page 17, lines 9-14, page 18, lines 22-24, page 19, line 17, and page 31, lines 12-26. Of newly added claims 119-143, claims 119-131 correspond to the elected Group I, drawn to GDF-8 polypeptides.

Objections to the Specification

The Examiner objected to the specification as not complying fully with the sequence listing requirements. Applicants have amended the specification accordingly and enclose a substitute Sequence Listing for inclusion into this application and a Statement pursuant to 37 C.F.R. § 1.821(f). Additionally, Applicants have submitted replacement drawings to include sequence identifiers. The new sequence listing incorporates sequences disclosed in the specification on pages 6, 18, 21, and 24 and in Figures 7 and 11. Applicants, thus, request that the Examiner withdraw this rejection.

Anticipation Rejection

The Examiner has rejected claims 1, 2, 4, 5, 15, 16, 19, and 20 under 35 U.S.C. § 102(a) as anticipated by WO 00/43781 (*Topouzis*). The Examiner alleges that *Topouzis* teaches GDF-8 from several species in Figure 11. It also allegedly teaches that GDF-8 has an inhibitory effect. Lastly, it allegedly discloses variants of the propeptide region, including altered proteolytic sites and altered glycosylation sites.

Applicants have canceled claims 1-20 and have added new claims directed to a particular mutated form of GDF-8 propeptide, which has unexpected beneficial properties, as discussed below. By initially prosecuting the subject matter of new claims 119- 141, Applicants do not acquiesce in the rejection of claims 1-20, and expressly reserve the right to pursue the subject matter of the cancelled claims in the future. *Topouzis* does not teach the claimed modified GDF-8 propeptide and thus cannot anticipate the invention as it is now claimed.

Obviousness Rejections

Topouzis

The Examiner has rejected claims 1-3 under 35 U.S.C. § 103(a) as allegedly obvious over *Topouzis*. The Examiner acknowledges that *Topouzis* fails to teach a GDF-8 inhibitor that is identical to SEQ ID NO:5 (human GDF-8 propeptide). The Examiner, however, believes that it would be obvious to create such a sequence with the N-terminal inhibiting sequence NENSE disclosed in *Topouzis* and the knowledge of the C-terminal proteolytic processing site that generates the proregion.

Applicants have canceled claims 1-3 and now claim a particular mutated form with unexpected beneficial properties, as discussed below. *Topouzis* does not teach or suggest this mutation. Thus, this rejection should be withdrawn.

Topouzis* and *Chang

The Examiner has rejected claims 6-14 under 35 U.S.C. § 103(a) as unpatentable over *Topouzis* in view of U.S. Patent No. 5,723,125 (*Chang*). The Examiner acknowledges that *Topouzis* does not teach the fusion of GDF-8 propeptide with IgG1 Fc or IgG4 Fc. The Examiner, however, asserts that *Chang* compensates for

this deficiency as it teaches that fusion of interferon with IgG1 or IgG4 prolongs the half-life of interferon.

As Applicants have canceled claims 6-14 and now claim a particular mutated form of GDF-8 propeptide with unexpected beneficial properties, as discussed below, this rejection should be withdrawn.

Topouzis* and *Sivam

The Examiner has rejected claims 6 and 17 under 35 U.S.C. § 103(a) as allegedly unpatentable over *Topouzis* in view of U.S. Patent No. 5,116,944 (*Sivam*). The Examiner acknowledges that *Topouzis* does not teach fusion of the GDF-8 propeptide with albumin. The Examiner, however, asserts that *Sivam* compensates for this deficiency as it teaches that fusion of proteins with albumin can prolong their half-lives and reduce toxicity.

As Applicants have canceled claims 6 and 17 and now claim a particular mutated form of GDF-8 propeptide with unexpected beneficial properties, as discussed below, this rejection should be withdrawn.

Topouzis* and *Davis

The Examiner has rejected claims 6 and 18 under 35 U.S.C. § 103(a) as allegedly unpatentable over *Topouzis* in view of U.S. Patent No. 4,179,337 (*Davis*). The Examiner acknowledges that *Topouzis* does not teach fusion of GDF-8 propeptide with polyethylene glycol (PEG). The Examiner, however, asserts that *Davis* compensates for this deficiency as it teaches that fusion of proteins with PEG can reduce immunogenicity.

As Applicants have canceled claims 6 and 18 and now claim a particular mutated form of GDF-8 propeptide with unexpected beneficial properties, as discussed below, this rejection should be withdrawn.

Unexpected Properties of Claimed Mutated GDF-8 Propeptide

Applicants enclose the Declaration of Neil M. Wolfman under 37 C.F.R. § 1.132 (Wolfman Declaration). This declaration presents evidence that mutated GDF-8 propeptides claimed in the newly added claims show unexpected beneficial properties when compared to wild-type GDF-8 propeptide. This declaration establishes that a GDF-8 propeptide with a mutation at an aspartate residue corresponding to Asp-99 in SEQ ID NO:1 (Asp-76 of SEQ ID NO:5) has beneficial properties. In particular, aspartate-to-alanine (D/A) propeptide mutant maintains potent inhibitory activity when compared to the wild-type protein. Wolfman Declaration, at ¶ 4-5.

The declaration also demonstrates increased *in vitro* stability to proteolytic cleavage by a protease present in the conditioned medium. Wolfman Declaration, at ¶ 7-8. Furthermore, it shows increases in *in vivo* biological activity due to the mutation in the propeptide sequence. Wolfman Declaration, at ¶ 9-13. In particular, this declaration presents data establishing that lower doses of the claimed mutated GDF-8 propeptide, administered over a shorter treatment period, yield greater gain in muscle mass as compared to the wild-type protein. For example, in the gastrocnemius muscle, essentially equivalent increases were seen with 50 mg/kg of wild-type GDF-8 Pro-Fc and only 1 mg/kg of mutated GDF-8 Pro-Fc. Furthermore, only 10 mg/kg of mutated GDF-8 Pro-Fc resulted in an 18% increase in muscle mass. In the quadriceps, only an

11% increase was shown with 50 mg/kg, while with the mutant treatment with 10 mg/kg resulted in a 21% increase in muscle mass. Wolfman Declaration, ¶ 11.

These studies demonstrate that GDF-8 propeptide fusion inhibits GDF-8 activity *in vivo* which leads to an increase in muscle mass. The D/A mutation in the proteolytic cleavage site of GDF-8 propeptide leads to increased stability of the protein *in vitro* and an increase in biological activity *in vivo*. This data also demonstrates that the claimed mutated GDF-8 propeptide exhibits unexpected properties compared to the wild-type protein. Wolfman Declaration, ¶ 12.

Therefore, Applicants assert that the claimed modified GDF-8 propeptides have unexpected beneficial properties when compared to prior art GDF-8 propeptide constructs. Applicants thus request that the Examiner withdraw the prior art rejections. *Topouzis* does not teach or suggest a GDF-8 propeptide with at least one mutation in the amino acid sequence at an aspartate residue corresponding to Asp-99 in SEQ ID NO:1. *Chang*, *Sivam*, and *Davis* do not cure this defect.

Definiteness Rejection

The Examiner has also rejected claims 1-20 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. The Examiner states that the claims are drawn to modified peptides, yet encompass SEQ ID NO:5, which is an unaltered fragment of GDF-8.

Applicants request that the Examiner withdraw the rejection as these claims have been canceled from the application.

Conclusion

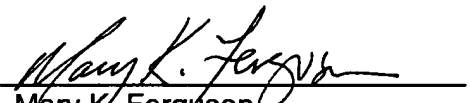
In view of the foregoing amendments and remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: February 20, 2004

By: 
Mary K. Ferguson
Reg. No. 51,675

Attachments:
Sequence Listing (paper and disk)
Statement under 37 CFR § 1.821(f)
Declaration under 37 CFR § 1.132
Replacement Drawing Sheets (3)
Request for Extension of Time

PATENT
Customer No. 22,852
Attorney Docket No. 08702.0100-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
WOLFMAN *et al.*	)	Group Art Unit: 1646
	)	
Application No.: 10/071,499	)	Examiner: Janet L. Andres
	)	
Filed: February 8, 2002	)	
	)	
For: MODIFIED AND STABLIZED GDF PROPEPTIDES AND USES THEREOF	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF NEIL M. WOLFMAN UNDER 37 C.F.R. § 1.132

I, Neil Wolfman, declare:

1. I am an inventor of the subject matter in application Serial No. 10/071,499.

2. I am a Director of Protein Biochemistry at Wyeth and have been employed at Wyeth (and its predecessor company, Genetics Institute) in various scientific capacities since 1984. I received my Ph.D. in Biophysical Chemistry from Cornell University in 1979, and my B.A. in Chemistry from New York University in 1974.

3. I have read and understood application Serial No. 10/071,499, including the claims as amended in the response filed herewith. The application, as amended, now claims an isolated modified GDF-8 propeptide having at least one mutation in the amino acid sequence at an aspartate residue corresponding to Asp-99 in SEQ ID NO:1. This mutation results in unexpected beneficial properties when compared to the wild-type GDF-8 propeptides, having the aspartate residue at position 99 in SEQ ID NO:1.

Biological Activity of Mutated GDF-8 Propeptides

4. We have evaluated a mutated GDF-8 propeptide-Fc fusion that falls within the scope of new claim 119 and have shown that it maintains biological activity when compared to the wild-type GDF-8 Pro-Fc fusion. We prepared a murine GDF-8 propeptide fused to the Fc region of an immunoglobulin (GDF-8 Pro-Fc) and a similar construct having a mutation from aspartate to alanine at the position corresponding to residue 99 in SEQ ID NO:1 (D/A GDF-8 Pro-Fc). We expressed both of these constructs in CHO/A2 cells that were stably transfected. We collected conditioned media from both transfected cell lines and purified the two GDF-8 propeptide constructs.

5. Pooled fractions of D/A GDF-8 Pro-Fc were quantitated by spectrophotometry and assayed for activity along with wild-type GDF-8 Pro-Fc fusion in the reporter gene assay as described in Example 3 of the specification. As shown in Figure 1, IC_{50} of D/A GDF-8 Pro-Fc is 0.3 nM indicating that the mutated propeptide has retained potent inhibitory (neutralizing) activity.

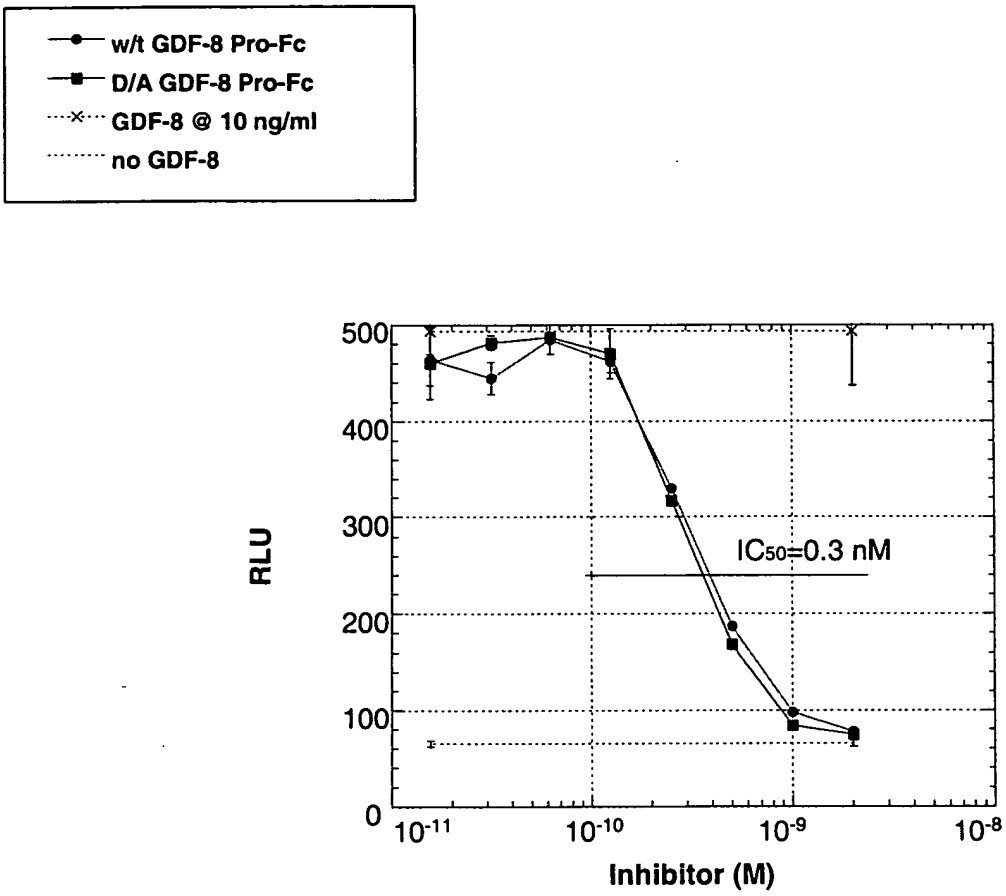
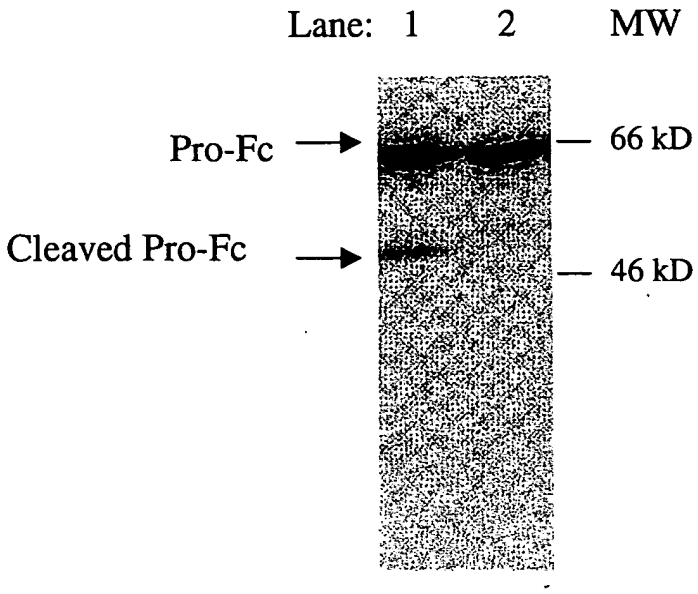


Fig. 1

6. This demonstrates that D/A-mutated GDF-8 propeptide maintains neutralizing activity.

***In Vitro* Stability of GDF-8 Pro-Fc Fusion Proteins**

7. We used the conditioned media collected from stable cell lines described above to evaluate the *in vitro* stability of the D/A mutant and wild-type GDF-8 Pro-Fc. Proteins were analyzed by SDS-PAGE under reducing conditions followed by Western blotting. Proteins were visualized using anti-murine IgG-HRP and a chemiluminescence detection system. The results of the Western blot are shown in Figure 2. Conditioned medium collected from the cell line expressing GDF-8 Pro-Fc (lane 1) shows two bands: 65 kD (intact Pro-Fc) and 50 kD (cleaved Pro-Fc) whereas only the 65 kD band (intact Pro-Fc) is seen in conditioned medium collected from the cell line expressing D/A-mutated GDF-8 Pro-Fc (lane 2). This proteolytic cleavage is attributed to a protease apparently present in the conditioned medium. Therefore, as a result of the D/A mutation, proteolytic cleavage of GDF-8 Pro-Fc was reduced or prevented as compared to the unmodified GDF-8 Pro-Fc.



Lane 1: wild-type GDF-8 Pro-Fc
Lane 2: D/A GDF-8 Pro-Fc

Fig. 2

8. This demonstrates that D/A GDF-8 Pro-Fc is less susceptible to proteolytic cleavage than the wild-type propeptide fusion construct.

Treatment of Mice with Wild-Type and Mutated GDF-8 Pro-Fc Fusion Proteins

9. The wildtype GDF-8 Pro-Fc fusion protein was tested in adult mice. Eight week old, adult, female BALB/c mice were randomized with respect to body weight and placed into groups of seven (except for the no treatment group, which had five mice). Mice were dosed twice weekly by IP injection with a total weekly dose of 0.5 mg/kg, 5 mg/kg, and 50 mg/kg (10 µg, 100 µg, or 1000 µg per animal) per animal for five weeks. Control injections were murine IgG2am at a molar equivalent to the high dose of GDF-8 Pro-Fc. At the end of the study, gastrocnemius and quadriceps were removed and weighed. Figures 3A and 3B show the mean tissue mass, with the error bars indicating the standard error of the mean. The asterisks indicate a statistically significant difference ($p<0.01$, Student's T test) when compared with the mice treated with the control protein, IgG2am. Blocking GDF-8 activity *in vivo* by IP injection of GDF-8 Pro-Fc at 50 µg/kg/week (treated mice) resulted in a 6.4% increase in gastrocnemius and an 11% increase in quadriceps muscle mass compared to control mice not receiving GDF-8 propeptide-Fc fusion protein. In summary, the GDF-8 propeptide blocked GDF-8 activity *in vivo* and led to a moderate increase in muscle mass of the gastrocnemious and quadriceps muscles compared to control mice.

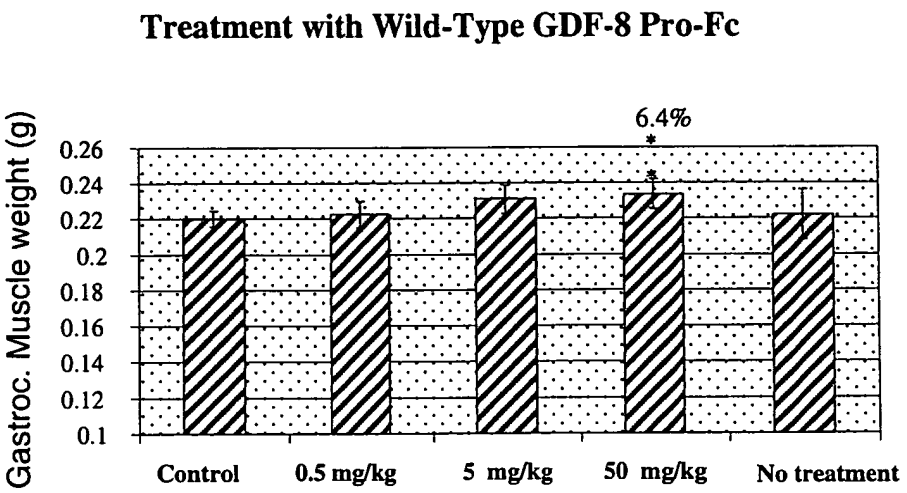


Fig. 3A

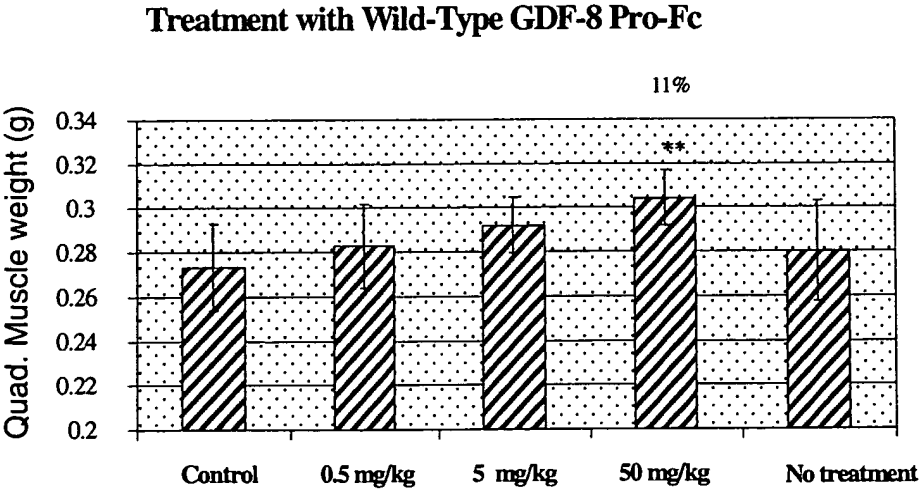


Fig. 3B

10. Similarly, the D/A-mutated GDF-8 propeptide-Fc fusion protein (D/A GDF-8 Pro-Fc) was tested *in vivo* in adult mice for increased efficacy which is measured by a change in muscle mass. Three-month-old female SCID mice were randomized with respect to body weight and placed into groups of eight. D/A GDF-8 Pro-Fc in PBS was injected into the mice by IP injection with 1 or 10 mg/kg weekly for four weeks. Mice injected with vehicle (PBS) were used as controls. At the end of the treatment, mice were sacrificed and gastrocnemius and quadriceps dissected and weighed. Figures 4A and 4B show the mean tissue mass, with the error bars indicating the standard error of the mean. The asterisks indicate a statistically significant difference ($p<0.01$, Student's T test) when compared with the control mice. Gastrocnemius and quadriceps weights were 18% (Fig. 4A) and 21% (Fig. 4B) greater, respectively, in the 10 mg/kg group as compared to the vehicle controls. There was a marginal increase (6%) in both muscles in the group treated at the dose of 1 mg/kg.

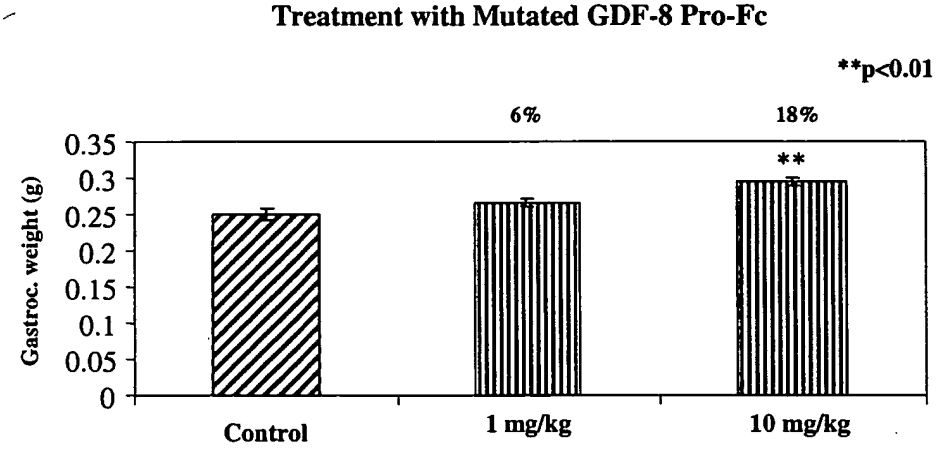


Fig. 4A

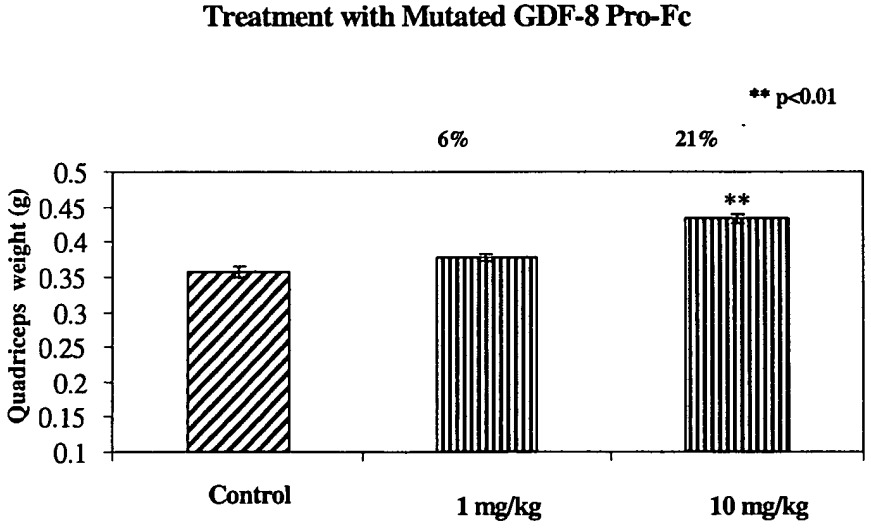


Fig. 4B

11. Therefore, compared to GDF-8 Pro-Fc, D/A GDF-8 Pro-Fc has much higher in vivo activity as indicated by muscle mass increase after treatment. For example, in the gastrocneumius muscle, essentially equivalent increases were seen with 50 mg/kg of wild-type GDF-8 Pro-Fc and only 1 mg/kg of mutated GDF-8 Pro-Fc. Furthermore, only 10 mg/kg of mutated GDF-8 Pro-Fc resulted in an 18% increase in gastrocnemius muscle weight. In the quadriceps, only an 11% increase was shown with 50 mg/kg, while the treatment with D/A GDF-8 Pro-Fc at 10 mg/kg resulted in a 21% increase in muscle weight.

12. These studies demonstrate that GDF-8 propeptide fusion inhibits GDF-8 activity *in vivo* which leads to an increase in muscle mass. Further, the D/A mutation in the proteolytic cleavage site of GDF-8 propeptide leads to increased stability of the protein *in vitro* and results in increased biological activity *in vivo*. This data also demonstrates that the claimed modified GDF-8 propeptides exhibit unexpected properties in view of the wild-type protein.

13. Therefore, I believe that the claimed invention was unexpected compared to prior art GDF-8 propeptide constructs.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 2/10/04

By: Neil M. Wolfman
Neil M. Wolfman, Ph.D.